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OBSERVATIONS ON THE SPREAD AND PERSISTENCE OF THE HEMOLYTIC STREPTOCOCCI PECULIAR TO SCARLET FEVER

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1. THE STREPTOCOCCI ISOLATED FROM ATTENDANTS AND SURROUNDINGS OF SCARLET FEVER PATIENTS

In previous articles I [1] have shown that the hemolytic streptococci isolated from early cases of scarlet fever and its complications belong to a distinct biologic group, apparently peculiar to scarlet fever, on account of their being specifically opsonified and agglutinated by the serum of a sheep immunized with a hemolytic streptococcus from scarlet fever. This serum also specifically protected mice against hemolytic streptococci from scarlet fever. Bliss,[2] reached similar conclusions from agglutination tests of streptococci from scarlet fever with immune rabbit serum as did also more recently Gordon.[3]

It seemed interesting to determine by opsonic and agglutination tests whether hemolytic streptococci from the rooms, eating utensils and attendants of diphtheria and scarlet fever patients belong to this same biologic group, and tests have been made of 20 strains of hemolytic streptococci isolated by Dr. W. J. Matousak [4] from the scarlet fever and the diphtheria rooms of the Durand Hospital. His results, reported elsewhere, were in brief as follows:

Hemolytic streptococci were isolated from the walls of diphtheria rooms once and from the nurses' shoes three times, but not from the fingernails, floor, door knobs or soap brushes.

Cultures were taken in rooms of scarlet fever patients, the throats of whom (in one instance also a leg abscess) harbored hemolytic streptococci specific for scarlet fever. Hemolytic streptococci were isolated from the fingernail of a nurse once, the outside of a face mask twice, the floor three times, the floor just outside once, the wall twice, the shoes of nurses three times, the cup, fork or spoon used by patients four times. The cultures from the door knobs, soap brushes, unused dishes and light buttons were negative.

All of the 20 strains of streptococci grew in chains in broth and produced a wide zone of hemolysis, from 2 to 4 mm., on goat blood agar plates after 24 hours' incubation. The strain from the fingernail

Received for publication March 19, 1921.

[1] J. A. M. A., 1920, 74, p. 1386, and 75, p. 1339.
[2] Bull. Johns Hopkins Hosp., 1920, 31, p. 173.
[3] Br. Med. J., 1921, 1, p. 632.
[4] Jour. Am. Med. Assn., 1921, 76, p. 1490.

produced large mucoid colonies, the others were small, round and grayish. All of the streptococci fermented lactose and salicin, but not mannite or inulin, and consequently would be classed as Streptococcus pyogenes.[5]

Opsonic and agglutination experiments were made with these strains of streptococci and the serum of a sheep which had been immunized with a hemolytic streptococcus isolated from the throat of a severe acute case of scarlet fever. Immunization had been carried on 8 months when these experiments were commenced. Some of the opsonic experiments were made first with fresh immune sheep serum. The strains were tested again with serum which had been stored for six months in the ice box and was still specific for hemolytic streptococci from scarlet fever. The agglutination experiments were almost all made with the stored serum.

For the opsonic experiments, the cocci were grown on blood agar for 24 hours and suspended in physiologic sodium chlorid solution. Normal sheep leukocytes, collected in 0.2% sodium citrate solution and washed once in salt solution were used. The serum, normal and immune, was heated for one-half hour at 56 C. to remove the thermolabile element, and then diluted with salt solution. The mixtures of diluted serum, leukocytes and coccal suspensions, equal parts, were incubated for 25 minutes, smears stained with carbolthionin, 50 polymorphonuclear leukocytes counted, and the number of cells taking part in phagocytosis noted. The point of opsonic extinction was determined by finding the dilution at which opsonification ceased. When the old stored serum was used it was necessary to activate it by fresh sheep serum, one part of fresh serum being added to one part each of diluted serum, leukocytes and coccal suspension.

In the agglutination tests cocci were grown from 24 to 48 hours in ascitic dextrose broth, one part of ascitic fluid to four parts of 1.0 % dextrose broth, P_H 7.8. The cultures were centrifuged, the supernatant fluid removed and the cocci washed once or twice in plain meat extract broth, P_H 7.8, and finally suspended in this medium. In the tests the serum dilutions were made with plain broth, the dilutions running from 1:10 to 1:800, and equal parts of bacterial suspension were added to each tube of diluted serum, and the mixture incubated for one hour at 55 C. Tubes containing suspended cocci in broth but without serum and also mixtures of cocci in normal sheep serum diluted from 1:10 to 1:400 or higher were included in each

[5] Holman, W. L.: Jour. Med. Res., 1916, 34, p. 377.

test. The immunizing streptococcus was tested in each experiment to serve as a standard. Later salt solution was substituted for the broth in making the dilutions and suspending the cocci and gave somewhat clearer agglutination. One lot of ascitic fluid with a specific gravity of 1.020 caused spontaneous clumping of the cocci and could not be used.

The 4 strains isolated from the shoes of nurses and the wall of the diphtheria room were neither opsonified nor agglutinated by the immune serum in higher dilutions than by normal sheep serum. Only 5 of the 16 strains isolated in the scarlet fever rooms were found to belong to the scarlet fever group of streptococci. One of these strains was isolated from a face mask and the others from the eating utensils (fork, spoon and cup) used by scarlet fever patients harboring hemolytic streptococci, which were specific for scarlet fever. The point of opsonin extinction for these streptococci varied from 1:40 to 1:240, the point of extinction for normal sheep serum being 1:3. The lower points of extinction were found when the stored serum was used. These streptococci were agglutinated at a dilution of 1:200 to 1:400. None were agglutinated by normal sheep serum.

These experiments indicate the value of face masks as a protection from pathogenic bacteria and the necessity of careful sterilization of eating utensils used by patients with infectious diseases. The results are in accord with those of Brown, Petroff and Pesquera,[6] Cummings, Spruit and Reuter,[7] and Saelhof and Heinekamp,[8] who came to the conclusion that eating utensils are a source of danger in the transference of tubercle bacilli and other pathogenic bacteria, unless properly cleansed. Cummings and his coworkers found that 87% of the eating utensils used by patients or healthy carriers of hemolytic streptococci were still contaminated with this organism after they were hand washed. Saelhof and Heinekamp isolated hemolytic streptococci, virulent for rabbits, from restaurant table ware in 6.35% of the articles examined.

2. ATYPICAL CASE OF SCARLET FEVER

I have isolated hemolytic streptococci (St. pyogenes) from the throats of 10 attendants at the Durand Hospital who had tonsillitis; the throats of 3 normal nurses, one a diphtheria nurse, the other two scarlet fever nurses; from the infected fingers of two interns; and from the cerebrospinal fluid in a case of meningitis following tonsillitis.

[6] Thirty-Fifth Annual Med. Report, Trudeau Sanatorium, 1919.
[7] The Military Surgeon, 1920, 2, p. 592.
[8] Am. Jour. Public Health, 1920, 10, p. 704.

Only 2 of these strains were opsonified and agglutinated by the immune sheep serum, the point of opsonic extinction being 1:80 and agglutination positive at 1:400 for both strains. There was neither opsonification nor agglutination with normal sheep serum. One strain was isolated from the throat of a maid with acute tonsillitis without an exanthem; the other from a scarlet fever nurse with a very red throat, a leukocytosis of 12,500, but no eruption and no rise in temperature. The nurse had had scarlet fever previously. These 2 cases would appear to be in the same class as 2 described by Bliss, who isolated hemolytic streptococci specific for scarlet fever from 2 patients exposed to scarlet fever, who had acute tonsillitis without exanthem.

Agglutination experiments with hemolytic streptococci isolated from patients with rash suggestive of scarlet fever have proved helpful in several cases in verifying a diagnosis of scarlet fever. A streptococcus from the discharging ear of a colored boy, entering the hospital with a scarcely visible eruption, was strongly agglutinated by the immune serum. A streptococcus from the throat of a nurse with an exanthem was not agglutinated and the rash was subsequently ascribed to antitoxic serum given 5 days previously.

A streptococcus from a patient with tonsillitis and a slight erythema was not agglutinated; further examination of the patient and the course of the disease indicated that the patient did not have scarlet fever. A streptococcus isolated from the discharging ear of a diphtheria patient was agglutinated; diffuse exanthem persisting for 5 days with subsequent desquamation accompanying the urticaria following the administration of antitoxin, indicated that the patient had scarlet fever also. The nurse in attendance contracted scarlet fever 3 days after she commenced nursing this patient.

A streptococcus isolated at necropsy from the lung of a measles patient with bronchopneumonia was agglutinated by the immune serum. It was considered that this patient probably had scarlet fever on account of the discharging ears, enlarged cervical glands, purulent infection of the accessory sinuses and a diffuse erythematous eruption, accompanying the measles rash.

Two cases are of interest because the agglutination experiments throw some light on the etiologic relation between the streptococci and the pathologic conditions. In one instance the hemolytic streptococci isolated from a vaccination wound of a patient with scarlet fever were not agglutinated, indicating that the two infections were coincident; in the other case, the hemolytic streptococci isolated from the

throat and lochia of a case of puerperal fever and scarlet fever were both agglutinated in high dilutions by the immune serum, and suggested that the same streptococcus was responsible for both infections.

In my previous experiments I found that hemolytic streptococci, peculiar to scarlet fever, generally disappeared from the throats of scarlet fever patients during the third and fourth weeks of the disease, and I concluded that the streptococci isolated from the throats at the onset of the attack were immunologically different from most of those obtained during convalescence. Further studies along this line seemed necessary. When possible, 4 colonies of hemolytic streptococci (Str. pyogenes) were isolated from the throat of the scarlet fever patient every other day for 25 days and tested for opsonins and agglutinins with the immune sheep serum. All of the strains isolated up to the 13th day of the disease were opsonified and agglutinated in high dilutions. On the 17th day one strain was isolated which was opsonified by the immune serum, the agglutination test not being made. The other strains isolated after the 13th day were neither opsonified nor agglutinated.

3. PERSISTENCE OF STREPTOCOCCUS PECULIAR TO SCARLET FEVER IN THE THROAT, EARS AND NOSE

In the original group of cases reported, 2 strains of hemolytic streptococci were isolated from patients with otitis media following scarlet fever in the 4th and 6th weeks. Neither strain belonged to the scarlet fever group of hemolytic streptococci. Since then another strain has been isolated from an ear, beginning to discharge 3 weeks after the onset of a case of probable scarlet fever. Three weeks later a hemolytic streptococcus was isolated and found not to belong to the scarlet fever group, being neither agglutinated nor opsonified by the immune sheep serum.

A strain of hemolytic streptococcus has been isolated during the 6th week from the purulent discharge of the nose of a scarlet fever patient, and was specifically agglutinated at a dilution of 1:200.

Streptococci from the discharging ear of a diphtheria patient, who had scarlet fever one month previously, was markedly agglutinated by the immune serum, which indicated that the ear infection was scarlatinal in origin.

These experiments suggest that while streptococci peculiar to scarlet fever are present as a rule from 3 to 5 weeks after the onset of the disease, they may persist much longer in patients with discharges, and that they disappear at the time the patient becomes non-infectious, according to clinical experience.

CONCLUSIONS

Hemolytic streptococci may be isolated from the floor and walls of rooms occupied by patients with scarlet fever and diphtheria, and from the fingernails, face masks and shoes of the attending nurses and from the eating utensils used by patients harboring hemolytic streptococci. Only 5 of 20 strains thus isolated were opsonified and agglutinated by the serum of a sheep immunized with a hemolytic streptococcus from scarlet fever and hence to be considered as specific for scarlet fever. Four of these strains were isolated from the eating utensils of scarlet fever patients and one from the face mask of the nurse in attendance. These results indicate the value of face masks and the necessity of disinfection of eating utensils used by patients with infectious diseases.

It would appear also that persons associated with scarlet fever patients may develop tonsillitis without an exanthem and harbor hemolytic streptococci which belong to the same biologic group as those isolated from typical cases of scarlet fever.

Agglutination of hemolytic streptococci from suspected cases of scarlet fever, by immune sheep serum specific for streptococci from scarlet fever, has proved helpful in diagnosis.

These results suggest, further, that while patients with scarlet fever generally rid themselves of hemolytic streptococci specific for scarlet fever in from 3 to 4 weeks, patients with discharges may retain them much longer, and that the streptococcus specific for scarlet fever disappears at the time when the patient becomes noninfectious, according to clinical experience.